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example, a segment of MMP-12 has recently been shown to have an antimicrobial effect (Houghton et al., 2009). Currently, the noncatalytic domains of MMPs are considered as being principally involved in substrate recognition and inhibitor binding, but we must now ask whether these domains can play roles in other biological processes. Studies showing noncatalytic roles for MMPs raise multiple questions for the field. (1) MMP activity has several control checkpoints including a requirement for proteolytic removal of a pro-domain to generate an active protease. Do the findings by Gonzalo et al. imply that noncatalytic functions of MMPs are less potent because the activation of the enzyme is no longer a required regulatory checkpoint? Or is an extra level of control provided by other players in these nonproteolytic events? (2) Development of MMP inhibitors has largely focused on blocking proteolysis, but are there scenarios wherein a different domain of the MMP regulates a biological function that promotes disease progression? How would this be targeted? (3) Do we need to reinterpret observations garnered from MMP null mice? We assume that phenotypic manifestations are due to the ablation of the catalytic activity of the MMP in question, but could the lack of other domains be contributing to the resultant phenotypes? Given this scenario, would it be valuable to compare catalytically dead MMP knockin mice with the total nulls?

Gonzalo et al. have added another layer of complexity to MMP research, but the field has the tools that will be needed to address the questions raised by these new insights and to take MMP research to new heights.

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Turning the Page on Epigenetic Bookmarks

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Cells remember established patterns of gene expression through rounds of cell division despite dynamic changes in genomic chromatin structure. Two recent studies in *Molecular Cell* and *Nature Medicine*, broadly impacting on epigenetic gene regulation and disease, address how cells remember and suggest that both histone methyltransferases and locus specific DNA binding proteins can mark transcribed genes for reactivation after mitosis.

The mammalian genome is packaged into condensed heterochromatin and more open, or accessible, euchromatin—chromatin structures that can determine in large part what genes may be expressed and hence the differentiated state of a specific cell. These patterns of chromatin structure are established in cells by proteins that modify histone tails through methylation, acetylation, and ubiquitination or that methylate DNA directly at CG dinucleotides. Such epigenetic modifications are thought to instill cellular memory so that specific patterns of gene expression are inherited during repeated rounds of cell division (Ringrose and Paro, 2007). Two recent studies from Blobel et al. (2009), published in *Molecular Cell*, and Verdeguer et al. (2009), published in *Nature Medicine*, identify two distinct factors that may mediate the cell's ability to maintain and propagate active epigenetic marks.

During early embryonic development, cell lineage specification and loss of pluripotency may be due to the establishment or maintenance of epigenetic marks—repressive marks at chromatin domains not expressed in subsequent derivatives within the lineage, activating marks at expressed genes, and bivalent marks at genes destined to be expressed later (Bernstein et al., 2006; Azuara et al., 2006). Once histone modifications are established on chromatin, they must be propagated during many cell divisions despite the dynamic structural changes

that the genome undergoes to allow for replication and transmission. Indeed, DNA replication must have access to the double helix and be able to unwind the strands. Moreover, during mitosis, the genome must compact into metaphase chromosomes that are then unpackaged in the daughter cells. Recent evidence points to the direct binding of the Polycomb repressive complex 2 (PRC2) to existing repressive histone H3 lysine 27 methyl (H3K27me) marks as a potential means of propagating the pattern of repressive modifications in replicating DNA (Hansen et al., 2008). This PRC2 binding is mediated by an interaction between the methylated histone H3 tail with the WD40 repeat domains of EED (Margueron et al., 2009), a component of PRC2 necessary for postgastrulation development and global H3K27 methylation. Surprisingly, EED binds to other Polycomb repressive marks, including H3K9me3 and H4K20me3, but not to histone methylation marks like H3K4me3 and H3K36me3, which are associated with active gene expression. Thus, by recognizing existing repressive marks, EED may recruit the PRC2 complex and the associated EZH2 histone methyltransferase to stimulate methyltransferase activity and create new repressive marks during DNA replication.

How active epigenetic marks are maintained and propagated remains more mysterious. Recently, two important papers (Blobel et al., 2009; Verdeguer et al., 2009) address how active gene expression patterns may be maintained during cell division and, more critically, how the loss of gene expression may be linked to cell division in a disease process. Blobel et al. (2009) investigated the genome-wide localization of MLL (KMT2A), a mammalian Trithorax homolog that methylates H3K4. In contrast to a previous report, the authors find MLL associated with mitotic chromosomes. Even more surprising was the reshuffling of MLL during mitosis to a different class of genes than in interphase nuclei, with more than a thousand new MLL target sites found exclusively on mitotic chromatin. The promoters of many genes that are highly transcribed during interphase are occupied by MLL during mitosis, even if they are not bound by MLL during interphase. These data led the authors to propose that MLL is needed to bookmark highly expressed genes such that reactivation after mitosis could progress. Consistent with this, MLL-deficient cells delayed reactivation of the genes normally occupied by MLL during mitosis but exhibited no change in the reactivation kinetics of interphase MLL targets, such as the Hox loci. This mitotic reshuffling also correlated with the localization of MLL complex proteins, such as RbBP5, Ash2L, and Menin, at mitotic chromatin targets, suggesting that H3K4 methylation occurs at these sites.

Even though MLL is required for timely reactivation of certain genes after mitosis, the overall levels of H3K4 methylation do not appear to be affected in the MLL-depleted cells. This may reflect the sensitivity of the assays and the fact that MLL2 (KMT2B) localizes to many of the same genes in interphase nuclei that MLL recognizes on mitotic chromatin. It is of note that the WD40 domain protein WDR5 is a component of multiple MLL complexes and was initially thought to bind to H3K4me2. Given the structural similarities between the WD40 β propeller domains of WDR5 and EED, it seems attractive to consider whether WDR5 provides a similar link to propagate H3K4me3 during replication as EED does for PRC2. However, WDR5 does not appear to bind H3K4 methylated residues with greater affinity relative to unmodified lysine, and it has higher affinity for arginine residues in the histone H3 tail and in the MLL protein itself (Trievel and Shilatifard, 2009). Still, the idea that MLL bookmarks certain loci during mitosis such that gene expression can be reactivated appropriately is appealing in its simplicity. It also begets more questions regarding the locus specificity of MLL relocalization and the DNA binding proteins that may drive this reshuffling.

One such DNA binding protein that appears to provide an epigenetic bookmark for gene expression is HNF-1β. Mutations in HNF-1β are associated with renal cystic disease and with maturity onset diabetes of the young type 5. Previously, HNF-1β was identified as a transcriptional regulator of many genes associated with polycystic kidney disease, including *Pkhd1* and *Pkd2*. Recent work from Verdeguer et al. (2009) now shows that a temporal knockout of *HNF-1β* in mice also results in a rapidly progressing cystic kidney disease if the gene is deleted at a time when renal epithelial cells are still undergoing rapid cell division. However, if *HNF-1β* is deleted 10 days after birth or later when most cells are quiescent, there is little evidence of cystic disease. This phenomenon might simply be explained by the fact that cells must be dividing in order to promote cyst development in these mutants. Although this is surely part of the story, more intriguing observations also came to light. If *HNF-1β* was deleted early, cysts formed and the expression of *Pkd2* was lost, consistent with previous reports of direct transcriptional regulation. However, if *HNF-1β* was deleted late in development, *Pkd2* expression was not affected, suggesting that *HNF-1β* is not simply a transactivator of *Pkd2*. Rather, *HNF-1β* must have an epigenetic affect on initiating *Pkd2* expression but is dispensable once activation is specified.

In a fully developed adult kidney, ischemia or nephrotoxic injury can kill proximal tubule cells and promote the regeneration of new epithelia during a recovery phase. In vivo fate mapping studies showed that the regenerated renal epithelia are derived from existing epithelia that had survived the initial injury (Humphreys et al., 2008). Verdeguer et al. (2009) used this property to test whether reentry into the cell cycle would impact epithelial cells lacking *HNF-1β*. After ischemia, mice with late-deleted *HNF-1β* developed cystic disease in recovering kidneys. More importantly, active epigenetic marks at the *Pkd2* promoter decreased and the expression of *Pkd2* was lost. These data point to an important role for *HNF-1β* in maintaining active epigenetic marks at the *Pkd2* locus during cell division. This locus specificity function during mitosis for the maintenance of active epigenetic marks or the inhibition of repressive epigenetic mark accumulation may extend to other genes.

Such bookmarking functions are likely to be essential for retaining the cellular memory of any dividing cell. Since their necessity may only be evident once cell division is activated, mutations in genes such as *HNF-1β* in adults may underlie a latent form of a disease that requires a second or even third hit to promote cell division and manifestation of the disease phenotype. Understanding how the targets of such bookmarks are reset during cell division may lead to new

treatments of diseases that are impacted by the loss of epigenetic maintenance.

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Dcr1 Tracked Down

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RNAi is essential for pericentromeric heterochromatic formation in *S. pombe*, and although Dcr1, the initiator protein of this process, has been biochemically well described, its subcellular localization has remained elusive. In this issue of *Developmental Cell*, Emmerth et al. now show that Dcr1 is dynamically shuttling between nucleus and cytoplasm, adding new insight into the subcellular mechanics of RNAi.

RNA interference (RNAi) is a mechanism that uses small RNAs as specificity factors to regulate gene expression. One of the best-studied small RNA species is the class of small interfering RNAs (siRNA). siRNAs are processed from long dsRNA molecules by dicer, an evolutionary well-conserved RNaseIII-like ribonuclease, containing additional functionalities like a helicase domain and two RNA binding domains: PAZ (Piwi Argonaute Zwille) and dsRBD (double-strand RNA binding domain). Processed siRNAs are loaded into a cytoplasmic or a nuclear effector complex called RNA-induced silencing complex (RISC) or RNA induced transcriptional silencing complex (RITS) respectively. In these complexes, siRNAs are bound by an Argonaute protein. Depending on the homology between siRNA and target RNA and the type of Argonaute, the target sequence can be silenced in two different ways: through posttranscriptional gene silencing (PTGS), which acts directly on the target RNA itself by target cleavage or translational inhibition, or through chromatin dependent gene silencing (CDGS), in which chromatin of the chromosomal locus producing the homologous sequence is remodeled into a repressive state (Carthew and Sontheimer, 2009; Moazed, 2009).

An organism that has specialized its RNAi machinery to a great extent to direct chromatin modification is *Schizosaccharomyces pombe* (*S. pombe*). In *S. pombe*, the formation of pericentromeric heterochromatin is a layered process in which first euchromatic histone modifications are removed, and next, Clr4 methylates H3K9 that is subsequently bound by Swi6. Biochemical studies have identified two distinct complexes that are essential for this heterochromatin formation. The Argonaute protein Ago1, the tryptophan GW-motif-containing protein Tas3, and the chromodomain protein Chp1 make up the RITS complex, which physically interacts with outer centromeric repeat transcripts in an siRNA-dependent manner. The RNA-directed RNA polymerase complex (RDRC), which physically interacts with RITS and amplifies the siRNA signal, has three core components: Rdp1 (an RNA-directed RNA polymerase), Cid12 (a polyadenylation polymerase), and the predicted helicase Hrr1. The interactions among RITS, RDRC, and the centromeric repeats are Dcr1 dependent, and Dcr1 itself has been reported to interact with RDRC (Colmenares et al., 2007; Moazed, 2009; Motamedi et al., 2004; Verdel et al., 2004). Previous reports based on Dcr1 overexpression studies reported counter-intuitive cytoplasmic localization (Carmichael et al., 2006). In this issue of *Developmental Cell*, Emmerth and colleagues readdress the question of the subcellular Dcr1 localization (Emmerth et al., 2010).

First, live cell imaging was performed on moderately expressed Dcr1-GFP fusion protein, revealing a predominantly nuclear localization. Interestingly, the nuclear localization was not diffuse, but colocalized with the nuclear pores in granule-like structures on the inner side of the nuclear membrane. These structures do not co-localize with RITS components or with chromatin, implying that siRNA generation and target recognition could be physically separated events. Interestingly in *Arabidopsis thaliana* (*A. thaliana*) and in the animal germline-specific Piwi pathway, the processing of CDGS-associated